

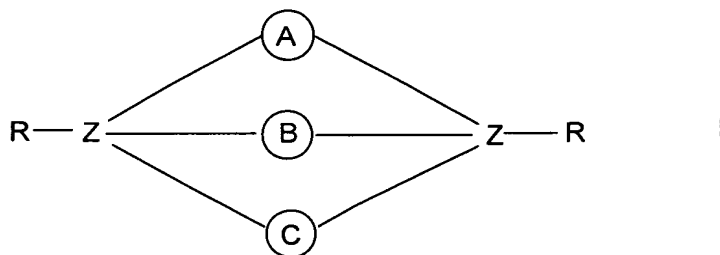


This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended): A process for constructing a signaling molecule by labeling a biological molecule, which can bind to a targeted partner, said process comprising covalently bonding to a biological molecule a labeling agent, wherein said labeling agent is a fluorescent conjugate comprising an oligonucleotide covalently bonded to a rare-earth metal cryptate,

wherein said rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound of formula I



wherein

Z is an atom with 3 or 4 valencies,

R is absence or is hydrogen, a hydroxy group, an amino group or a hydrocarbon-based radical, and

the divalent radicals (A) , (B) and (C) , are, independently of each other, hydrocarbon-based chains which optionally contain one or more hetero atoms and are optionally interrupted with a hetero macrocycle,

wherein at least one of the radicals (A) , (B) and (C) , also comprises at least one molecular unit, said molecular unit having a triplet energy which is greater than that of the emission level of the complexed rare-earth metal ion.

2. (Previously Presented): The process as claimed in claim 1, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester bonds.

3. (Previously Presented): The process as claimed in claim 1, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units or of analogous units of nucleotides modified on the sugar or on the base and bonded to one another via natural phosphodiester internucleotide bonds, wherein some of the internucleotide bonds are optionally replaced with phosphonate, phosphoramidate or phosphorothioate bonds.

4. (Previously Presented): The process as claimed in claim 1, wherein the oligonucleotide consists of a chain comprising both ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester bonds and analogous units of nucleosides bonded to one another via amide bonds.

5. (Previously Presented): The process as claimed in claim 1, wherein the oligonucleotide consists of ribonucleotide or deoxyribonucleotide units, wherein one of said units comprises a functional group selected from NH₂, COOH, CHO, OH, SH, halide, sulfonate, epoxide, and maleimide, introduced onto or generated on said one of said units, or the functional group is introduced using a spacer arm bonded to the terminal phosphate group in the 3' or 5' position.

6. (Previously Presented): The process as claimed in claim 5, wherein said unit is the 5' terminal unit or 3' terminal unit.

7. (Currently Amended): The process as claimed in claim 1, wherein the oligonucleotide comprises a chain of 5 to 50 nucleotides or a chain of 5 to 50 units containing nucleotides, and nucleotide analogs, or nucleoside analogs, or combinations thereof.

8. (Previously Presented): The process as claimed in claim 1, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester bonds and of analogous units of nucleosides bonded to one another via amide bonds, said oligonucleotide comprising at least 5 phosphodiester internucleotide bonds at the end bonded to the cryptate.

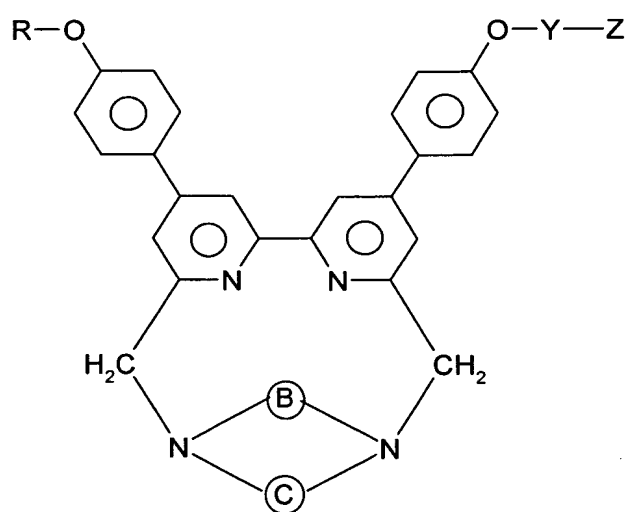
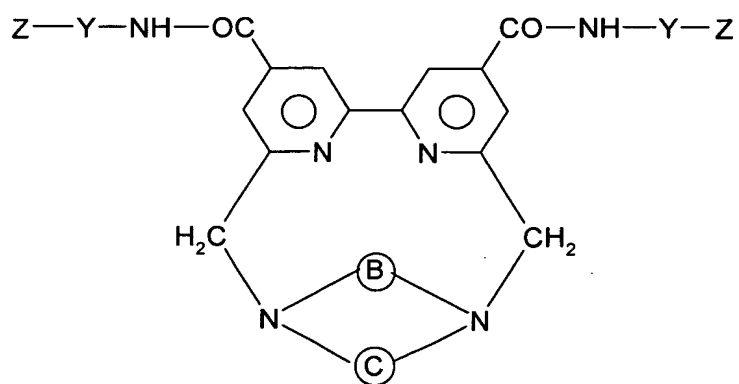
9. (Currently Amended): The process as claimed in claim 1, wherein the rare-earth metal cryptate is directly bonded covalently to the oligonucleotide ~~either directly or via a spacer arm~~.

10. (Cancelled):

11. (Currently Amended): The process as claimed in claim 1 ~~to~~, wherein the rare-earth metal cryptate consists of a rare-earth metal salt complexed with one of the macrocyclic or macropolycyclic compounds selected from the following compounds:

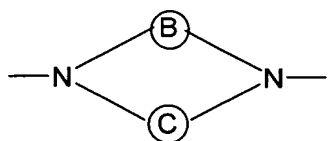
2.2.phenanthroline, 2.2.phenanthroline amide, 2.2.anthracene, 2.2.anthracene amide, 2.2.biisoquinoline, 2.2.biphenyl-bis-pyridine, 2.2.bipyridine, 2.2.bipyridine amide, trisbipyridine, trisphenanthroline, phenanthrolinebisbipyridine, biisoquinolinebisbipyridine, bisbipyridine diphenylbipyridine, and macropolycyclic compounds comprising a molecular unit chosen from bipyrazines, bipyrimidines and nitrogen-containing heterocycles comprising N-oxide groups.

12. (Previously Presented): The process according to claim 1, wherein the rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound corresponding to one of the formulae II or III below:

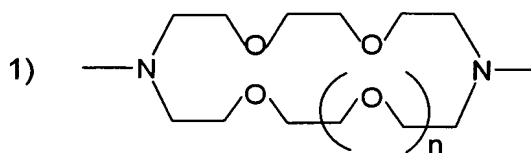


in which:

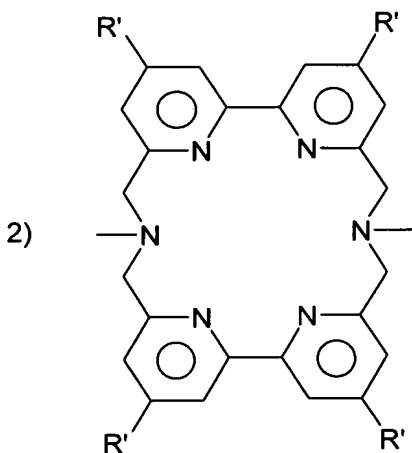
- the ring of formula



is one of the following rings:



wherein n is 0 or 1,



Y is a spacer group or spacer arm which is a divalent organic radical chosen from:

linear or branched C_1 or C_{20} alkylene optionally containing one or more double bonds and/or optionally containing one or more hetero atoms or one or more carbamoyl or carboxamido group(s),

C_5 to C_8 cycloalkylene, and

C_6 to C_{14} arylene,

said alkylene, cycloalkylene or arylene are in each case optionally substituted with alkyl, aryl or sulfonate groups;

Z is a functional group capable of bonding covalently to a biological substance;

R is methyl or -Y-Z;

R' is hydrogen, -COOR'', or -CO-NH-Y-Z;

R'' is C_1 to C_{10} alkyl.

13. (Currently Amended): The process as claimed in claim 1, wherein the rare-earth metal cryptate is bonded to the oligonucleotide via a spacer arm, wherein said spacer arm consists is consisting of a divalent organic radical chosen from:

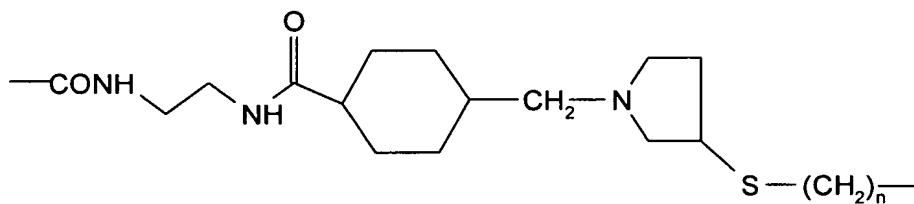
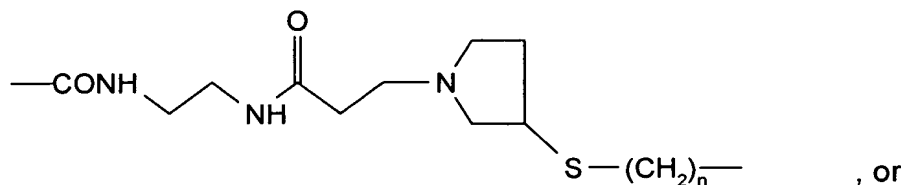
C₁-C₂₀ linear or branched alkylene optionally containing one or more double bonds or triple bonds and/or optionally containing one or more hetero atoms or one or more carbamoyl or carboxamino group(s);

C₅-C₈ cycloalkylene; and

C₆-C₁₄ arylene;

wherein said alkylene, cycloalkylene or arylene is in each case optionally substituted with alkyl, aryl or sulfonate groups.

14. (Currently Amended): The process as claimed in claim 13, wherein said spacer arm is:



wherein

n is 2 to 6; or

the spacer arm is $\text{---CONH---(CH}_2\text{)}_6\text{---}$;

wherein attachment to the cryptate occurs via the group ---CONH .

15. (Previously Presented): The process as claimed in claim 1, wherein the rare-earth metal cryptate is a europium cryptate.

16. (Previously Presented): The process as claimed in claim 15, wherein said europium cryptate is Eu trisbipyridine or Eu[bisdiethoxybipyridine.bipyridine].

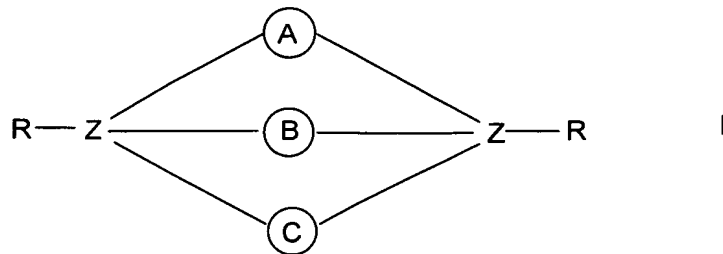
17. (Previously Presented): A fluorescence assay method for detecting an analyte comprising providing a measuring medium containing a sample to be tested for the presence of said analyte, wherein said measuring medium contains at least one fluorescent label, wherein said at least one fluorescent label is a fluorescent conjugate according to claim 20.

18. (Canceled)

19. (Previously Presented): The method as claimed in claim 1, wherein said measuring medium further comprises an acceptor fluorescent compound.

20. (Currently Amended): A conjugate comprising:
(1) a rare-earth metal cryptate;
(2) an oligonucleotide; and
(3) a biological molecule having a recognition role and which can bind to a partner,
wherein said cryptate, oligonucleotide, and biological molecule are linked by covalent bonds,

wherein said rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound of formula I



wherein

Z is an atom with 3 or 4 valencies,

R is absence or is hydrogen, a hydroxy group, an amino group or a hydrocarbon-based radical, and

the divalent radicals (A) , (B) and (C) , are, independently of each other,
hydrocarbon-based chains which optionally contain one or more hetero atoms and are
optionally interrupted with a hetero macrocycle,

wherein at least one of the radicals (A) , (B) and (C) , also comprises at least one
molecular unit, said molecular unit having a triplet energy which is greater than that of the
emission level of the complexed rare-earth metal ion.

21. (Previously Presented): The conjugate according to claim 20, wherein the biological molecule is one member of a pair molecules capable of binding specifically to one another.

22. (Previously Presented): The conjugate as claimed in claim 20, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester bonds.

23. (Previously Presented): The conjugate as claimed in claim 20, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units or of analogous units of nucleotides modified on the sugar or on the base and bonded to one another

via natural phosphodiester internucleotide bonds, wherein some of the internucleotide bonds are optionally replaced with phosphonate, phosphoramidate or phosphorothioate bonds.

24. (Previously Presented): The conjugate as claimed in claim 20, wherein the oligonucleotide consists of a chain comprising both ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester bonds and analogous units of nucleosides bonded to one another via amide bonds.

25. (Previously Presented): The conjugate as claimed in claim 20, wherein the oligonucleotide consists of ribonucleotide or deoxyribonucleotide units, wherein one of said units comprises a functional group selected from NH₂, COOH, CHO, OH, SH, halide, sulfonate, epoxide, and maleimide, introduced onto or generated on said one of said units, or the functional group is introduced using a spacer arm bonded to the terminal phosphate group in the 3' or 5' position.

26. (Previously Presented): The conjugate as claimed in claim 25, wherein said unit is the 5' terminal unit or 3' terminal unit.

27. (Currently Amended): The conjugate as claimed in claim 20, wherein the oligonucleotide comprises a chain of 5 to 50 nucleotides or a chain of 5 to 50 units containing nucleotides, and nucleotide ~~analogs~~, or nucleoside analogs, or combinations thereof.

28. (Previously Presented): The conjugate as claimed in claim 20, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester bonds and of analogous units of nucleosides bonded to one another via amide bonds, said oligonucleotide comprising at least 5 phosphodiester internucleotide bonds at the end bonded to the cryptate.

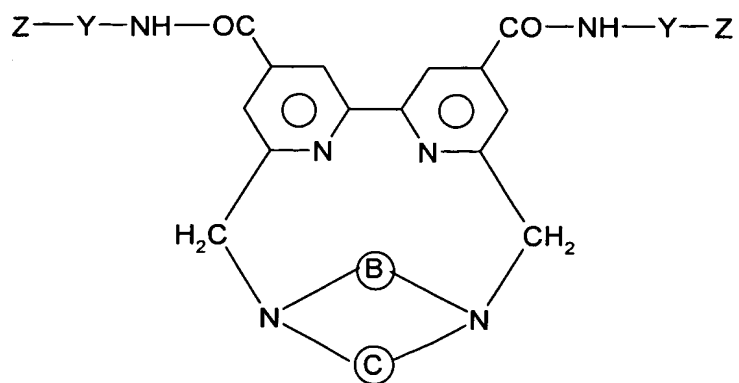
29. (Currently Amended): The conjugate as claimed in claim 20, wherein the rare-earth metal cryptate is directly bonded covalently to the oligonucleotide ~~either directly or via a spacer arm~~.

30. (Cancelled):

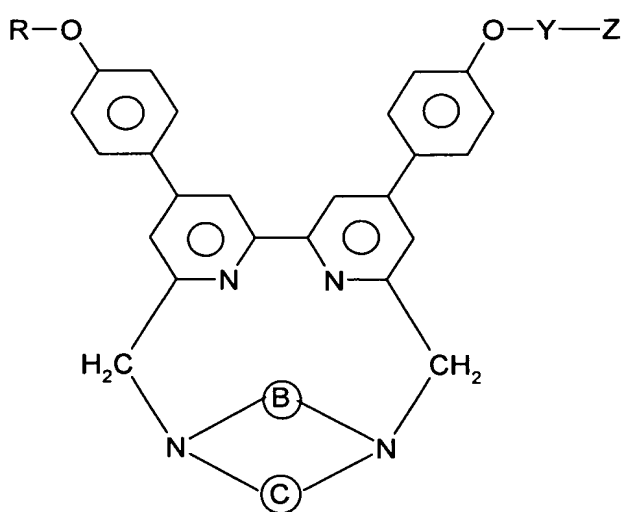
31. (Currently Amended): The conjugate as claimed in claim ~~20~~ 30, wherein the rare-earth metal cryptate consists of a rare-earth metal salt complexed with one of the macrocyclic or macropolycyclic compounds selected from the following compounds:

2.2.phenanthroline, 2.2.phenanthroline amide, 2.2.anthracene, 2.2.anthracene amide, 2.2.biisoquinoline, 2.2.biphenyl-bis-pyridine, 2.2.bipyridine, 2.2.bipyridine amide, trisbipyridine, trisphenanthroline, phenanthrolinebisbipyridine, biisoquinolinebisbipyridine, bisbipyridine diphenylbipyridine, and macropolycyclic compounds comprising a molecular unit chosen from bipyrazines, bipyrimidines and nitrogen-containing heterocycles comprising N-oxide groups.

32. (Previously Presented): The conjugate as claimed in claim 20, wherein the rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound corresponding to one of the formulae II or III below:



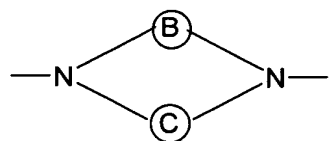
II



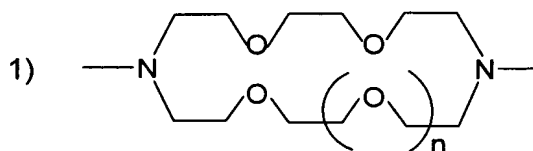
III

in which:

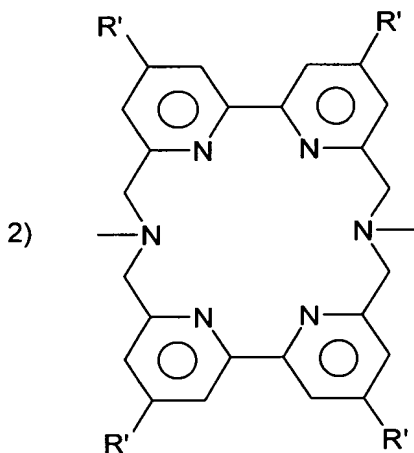
- the ring of formula



is one of the following rings:



wherein n is 0 or 1,



Y is a spacer group or spacer arm which is a divalent organic radical chosen from:

linear or branched C₁ or C₂₀ alkylene optionally containing one or more double bonds and/or optionally containing one or more hetero atoms or one or more carbamoyl or carboxamido group(s),

C₅ to C₈ cycloalkylene, and

C₆ to C₁₄ arylene,

said alkylene, cycloalkylene or arylene are in each case optionally substituted with alkyl, aryl or sulfonate groups;

Z is a functional group capable of bonding covalently to a biological substance;

R is methyl or -Y-Z;

R' is hydrogen, -COOR'', or -CO-NH-Y-Z;

R'' is C₁ to C₁₀ alkyl.

33. (Previously Presented): The conjugate as claimed in claim 20, wherein the rare-earth metal cryptate is bonded to the oligonucleotide via a spacer arm, wherein said spacer arm is a divalent organic radical chosen from:

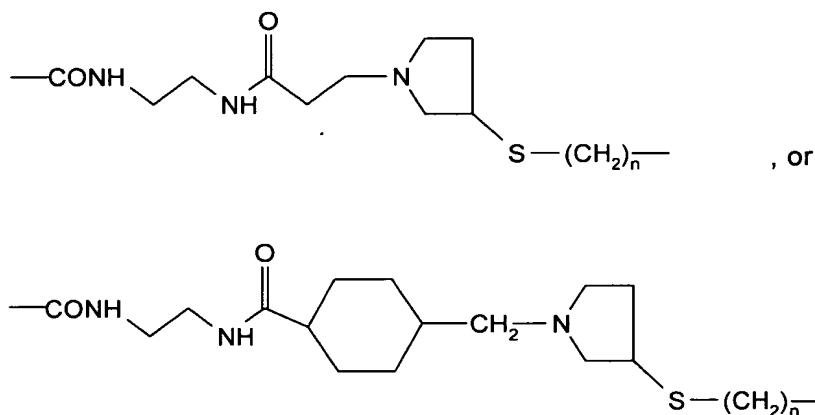
C_1 - C_{20} linear or branched alkylene optionally containing one or more double bonds or triple bonds and/or optionally containing one or more hetero atoms or one or more carbamoyl or carboxamino group(s);

C_5 - C_8 cycloalkylene; and

C_6 - C_{14} arylene;

wherein said alkylene, cycloalkylene or arylene is in each case optionally substituted with alkyl, aryl or sulfonate groups.

34. (Previously Presented): The conjugate as claimed in claim 33, wherein the spacer arm is:



wherein n is 2 to 6; or

the spacer arm is $\text{---CONH---(CH}_2\text{)}_6\text{---}$;

wherein attachment to the cryptate is via the group ---CONH .

35. (Previously Presented): The conjugate as claimed in claim 20, wherein the rare-earth metal cryptate is a europium cryptate.

36. (Previously Presented): The conjugate as claimed in claim 35, wherein said europium cryptate is Eu trisbipyridine or Eu [bisdiethoxybipyridine.bipyridine].

37. (Previously Presented): The conjugate as claimed in claim 21, wherein the biological molecule is a cellular receptor, an antigen, an antibody or a nucleic acid.

38. (Previously Presented): The conjugate as claimed in claim 32, wherein the R" alkyl group is a methyl, ethyl or tert-butyl group.

39. (Previously Presented): The conjugate as claimed in claim 33, wherein said one or more hetero atoms is oxygen, nitrogen, sulfur, or phosphorus.

40. (Previously Presented): The process according to claim 12, wherein R" is a methyl, an ethyl or a tert-butyl group.

41. (Canceled)

42. (Canceled)

43. (Canceled)

44. (Canceled)

45. (Canceled)

46. (Canceled)

47. (Previously Presented): The process according to claim 1, wherein said rare-earth metal cryptate is bonded to said oligonucleotide via a spacer arm, where in said spacer arm is a divalent organic radical chosen from:

a C₁-C₂₀ linear or branched alkylene optionally containing one or more double bonds or triple bonds and/or optionally containing one or more hetero atoms selected from oxygen, nitrogen, sulfur, and phosphorus or one or more carbamoyl or carboxamino group(s);

a C₅-C₈ cycloalkylene; and

C₆-C₁₄ arylene,

wherein in each case said alkylene, cycloalkylene or arylene is optionally substituted by alkyl, aryl or sulfonate groups.

48. (Previously Presented): The process as claimed in claim 3, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via natural phosphodiester internucleotide bonds, wherein some of the internucleotide bonds are replaced with phosphonate, phosphoramidate or phosphorothioate bonds.

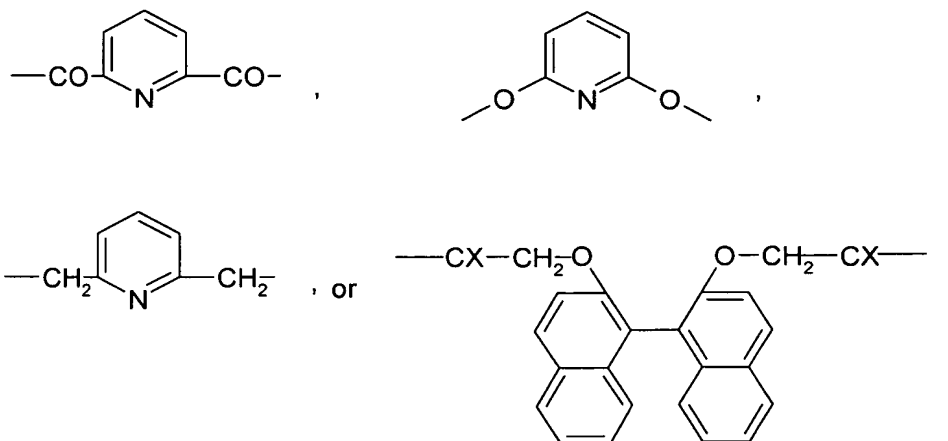
49. (Cancelled):

50. (Currently Amended): The process as claimed in claim 19, wherein the rare-earth metal cryptate is bonded covalently to the oligonucleotide via a spacer arm.

51. (Previously Presented): The process as claimed in claim 10, wherein at least one of the radicals (A), (B) and (C), consists essentially of a molecular unit having a triplet energy which is greater than that of the emission level of the complexed rare-earth metal ion.

52. (Previously Presented): The process according to claim 10, wherein said molecular unit is phenanthroline, anthracene, benzene, naphthalene, biphenyl, terphenyl, azobenzene, azopyridine, pyridine, bipyridine, bisquinoline, -C₂H₄-X₁-C₆H₄-X₂-C₂H₄-, or C₂H₄-X₁-CH₂-C₆H₄-CH₂-X₂-C₂H₄-, and

X₁ and X₂ are each O, N, or S, or
said molecular unit is



and X is oxygen or hydrogen.

53. (Previously Presented): The conjugate according to claim 20, wherein said rare-earth metal cryptate is bonded to said oligonucleotide via a spacer arm, where in said spacer arm is a divalent organic radical chosen from:

a C₁-C₂₀ linear or branched alkylene optionally containing one or more double bonds or triple bonds and/or optionally containing one or more hetero atoms selected from oxygen, nitrogen, sulfur, and phosphorus or one or more carbamoyl or carboxamino group(s);

a C₅-C₈ cycloalkylene; and

C₆-C₁₄ arylene,

wherein in each case said alkylene, cycloalkylene or arylene is optionally substituted by alkyl, aryl or sulfonate groups.

54. (Previously Presented): The conjugate as claimed in claim 23, wherein the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one

another via natural phosphodiester internucleotide bonds, wherein some of the internucleotide bonds are replaced with phosphonate, phosphoramidate or phosphorothioate bonds.

55. (Cancelled):

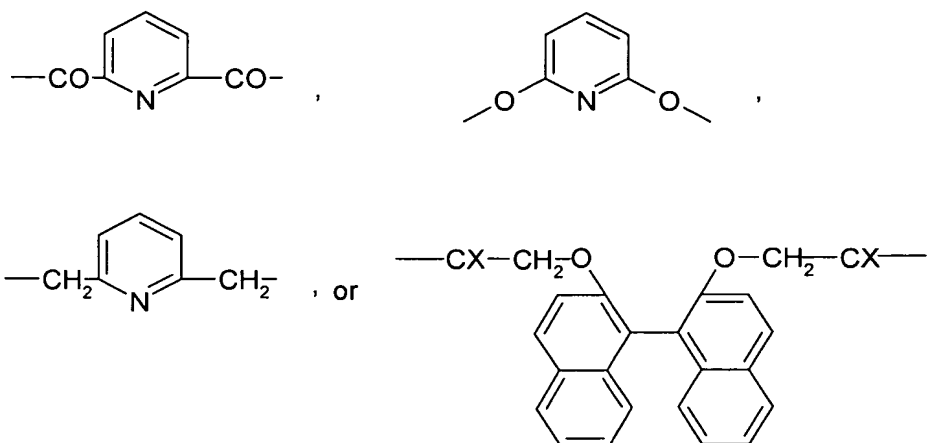
56. (Currently Amended): The conjugate as claimed in claim 20 ~~29~~, wherein the rare-earth metal cryptate is bonded covalently to the oligonucleotide via a spacer arm.

57. (Previously Presented): The conjugate as claimed in claim 30, wherein at least one of the radicals (A) , (B) and (C) , consists essentially of a molecular unit having a triplet energy which is greater than that of the emission level of the complexed rare-earth metal ion.

58. (Previously Presented): The conjugate according to claim 30, wherein said molecular unit is phenanthroline, anthracene, benzene, naphthalene, biphenyl, terphenyl, azobenzene, azopyridine, pyridine, bipyridine, bisquinoline, -C₂H₄-X₁-C₆H₄-X₂-C₂H₄-, or C₂H₄- X₁-CH₂-C₆H₄-CH₂-X₂-C₂H₄-, and

X₁ and X₂ are each O, N, or S, or

said molecular unit is



and X is oxygen or hydrogen.

59. (Previously Presented): The process according to claim 1, wherein the oligonucleotide is an oligodeoxynucleotide modified at its 5' end with an aminohexyl group for binding to said cryptate, and modified at 3' end with a structure containing a maleimide group for binding to the biological molecule.

60. (Previously Presented): The conjugate according to claim 20, wherein the oligonucleotide is an oligodeoxynucleotide modified at its 5' end with an aminohexyl group for binding to said cryptate, and modified at 3' end with a structure containing a maleimide group for binding to the biological molecule.

61. (Previously Presented): The process according to claim 1, wherein the oligonucleotide is a thiol-oligonucleotide wherein the thiol function is introduced at the 5' end of the oligonucleotide in the form of a disulfide bond.

62. (Previously Presented): The conjugate according to claim 20, wherein the oligonucleotide is a thiol-oligonucleotide wherein the thiol function is introduced at the 5' end of the oligonucleotide in the form of a disulfide bond.